

Functional Evidence of Persistent Airway Obstruction in Rats Following a Two-Hour Inhalation Exposure to Methyl Isocyanate

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Pulmonary function was assessed in male, F344 rats 1,2,4,7, and 13 weeks after a single 2-hr exposure to 0, 3, 10, or 30 ppm methyl isocyanate. No significant changes were observed in the rats exposed to 3 ppm through 13 weeks. Diffusing capacity (DL_{CO}), quasistatic lung compliance, and homogeneity of ventilation, as determined by multibreath nitrogen washout, were depressed in the rats exposed to 10 and 30 ppm by 1 week after exposure. None of the rats exposed to 30 ppm survived beyond 1 week. By 13 weeks, dramatic increases in lung volumes were observed in the rats exposed to 10 ppm, while DL_{CO} and lung compliance were only mildly affected. However, volume-specific DL_{CO} and compliance were depressed in the rats exposed to 10 ppm, suggesting that lung hyperinflation or other compensatory means of increasing lung size occurred in response to the methyl isocyanate-induced lung lesion. This group also exhibited increased expiratory times during tidal breathing and severely impaired distribution of ventilated air. Collectively, these results suggest the development and likely progression of a severe, obstructive airway lesion with associated gas trapping, and the existence of a pronounced concentration-response relationship between 3 and 10 ppm methyl isocyanate exposures.

Introduction

Methyl isocyanate (MIC) is a highly reactive chemical used in the production of various widely used carbamate pesticides. In December 1984, a large quantity of MIC was accidentally released into the atmosphere over Bhopal, India, killing or injuring several hundred thousand individuals. The role of this chemical as a pulmonary toxicant, not only to those who were exposed and may or may not recover, but also to those living in areas where the potential for exposure exists, has stimulated considerable scientific investigation.

MIC has been shown to be severely irritating to mucous membranes (1), causing marked bronchospasm and asthmatic breathing when inhaled (2). Recent studies have demonstrated that MIC is a potent sensory and pulmonary irritant in mice (3). Additionally, reports from Bhopal indicate that many survivors of the MIC release display symptoms of lung disease with both restrictive and obstructive components (4). However, a

comprehensive evaluation of the effects after exposure of MIC on pulmonary function has not been performed.

In an attempt to ascertain the functional sequelae of a single MIC exposure in the mammalian lung, we measured lung function in rats periodically for 3 months after exposure using a battery of pulmonary function tests.

Methods

Animals and Exposures

Male, 6- to 10-week-old F344 rats (Charles River, Kingston, NY) were divided into four treatment groups: one filtered-air control group, and three groups that received a single, 2-hr exposure to 3, 10, or 30 ppm MIC on April 27, 1985. Specific details of the exposure system and these particular exposures are reported elsewhere (5). After exposure, the animals were transferred to standard plastic holding cages and given food and water *ad libitum* until testing.

Pulmonary Function

Pulmonary function was evaluated 1, 2, 4, 7, and 13 weeks after exposure. The techniques used to measure lung function were similar to those previously reported (6,7). Before testing began, the animals were anesthetized with sodium pentobarbital (50 mg/kg body weight,

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IP) and tracheostomized. An appropriately sized (14–16 G) Luer stub adapter (Becton Dickinson, Rutherford, NJ) was then inserted and tied into the trachea.

For lung measurements, the animals were placed in an acrylic whole-body plethysmograph with a three-position slide valve (dead space 0.3 mL) that facilitated switching between two gas sources and a pressure transducer. End expiratory volume (EEV) was calculated from airway and plethysmographic recordings by using an application of Boyle's Law or computed by using measured nitrogen dilution. Vital capacity (VC) was measured between airway pressures of -15 and $+30$ cm H₂O. Single-breath diffusing capacity to carbon monoxide (DL_{co}) and residual volume (RV) were measured by using gas dilution techniques previously described (8). Total lung capacity (TLC) was computed as the sum of VC and RV. Respiratory system compliance (C_{rs}) was calculated by a nonlinear least-squares fit of the deflation portion of the quasi-static pressure-volume curve (9). A multiple-breath nitrogen washout maneuver at fixed tidal volume and frequency was performed by calculating the slope of the log end-tidal nitrogen concentration vs. breath number (N₂ slope) while the animal was ventilated with 100% oxygen.

After completion of the pulmonary function measurements, the animals were exsanguinated, the lungs removed, and the heart and mediastinal tissue dissected. After measuring the wet lung weight, the lungs were dried for 24 hr at 40°C at an inflation pressure of 30 cm H₂O. Dry lung weights were then recorded and percent lung water was calculated as

$$\% = \left[\frac{\text{Wet lung weight} - \text{dry lung weight}}{\text{Wet lung weight}} \right] \times 100$$

Data Collection

Analog output from the individual conditioned transducer signals was digitized and stored on floppy disks using a PDP 11/23+ microcomputer system (Digital Equipment Corporation, Maynard, MA). The digitized data were analyzed by using Fortran 77 programs developed in this laboratory. Further statistical analyses employed the SAS statistical package.

Statistics

The analysis of covariance (ANCOVA) was used to examine the effects of exposure on the individual pulmonary function parameters over time. Where no time trend existed, the data were analyzed by use of a one-way analysis of variance (ANOVA) and William's subtest to determine the lowest effective MIC concentration. If a time trend was observed, an appropriate regression model (linear or quadratic) was fit, and tests of parallelism among the concentration levels were performed. If the treatment regression models were parallel, differences among concentration levels were determined using a least significant differences test. When

models were not parallel, the slopes for each concentration level were tested for equality to one another using *t*-tests.

Additionally, one-way ANOVAs were performed at 1 week after exposure. Because the animals exposed to 30 ppm died soon after this time point, it was impossible to perform the time-trend analyses described above. All data presented in the accompanying table and figures are means \pm SEM, where the asterisk (*) denotes significant difference from control ($p < 0.05$).

Results and Discussion

Assessment of lung function in the rats exposed to 30 ppm MIC is reported only for 1 week after exposure, as none of these animals survived to the 2-week time-point. There were no deaths in the 0-, 3-, or 10-ppm groups.

Body weights were depressed by 33% at 1 week after exposure in rats exposed to 30 ppm MIC, and by an average of 14% in the animals exposed to 10 ppm at all time points. Several measures of lung volume were dramatically increased by 10-ppm MIC exposure. TLC was increased to 120% of control 4 weeks after exposure and to 140% of control by 13 weeks (Fig. 1). Residual volume increased to nearly three times control at 1 week after exposure and remained elevated during each examination after exposure (Fig. 2). EEV, as computed by Boyle's law, increased to 180% of control at 1 week after exposure and remained markedly elevated through 13 weeks (Fig. 3A). VC was only mildly affected by MIC exposure.

DL_{co}, which serves as an index of the efficiency of alveolar gas diffusion, was significantly depressed at 1 week following exposure in the 30-ppm group. Similarly, DL_{co} in the rats exposed to 10 ppm was also depressed at 1 and 2 weeks after exposure. Thereafter,

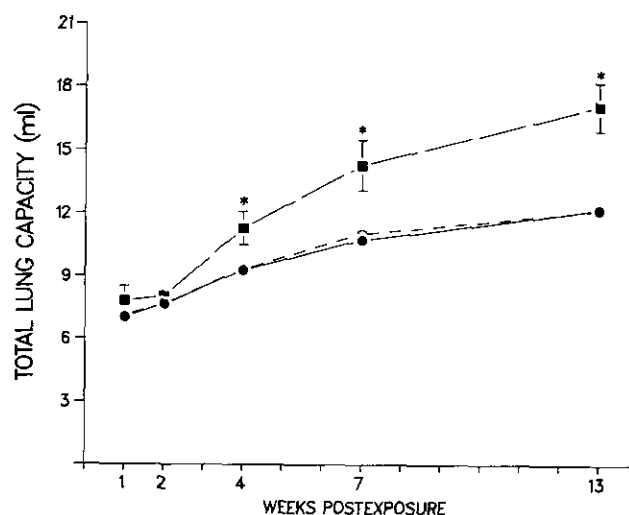


FIGURE 1. Total lung capacity in rats exposed to (●) 0 ppm, (○) 3 ppm, (■) 10 ppm, or 30 ppm (results identical to rats exposed to 10 ppm at 1 week) MIC for 2 hr. The asterisk (*) denotes significant difference ($p < 0.05$) from control.

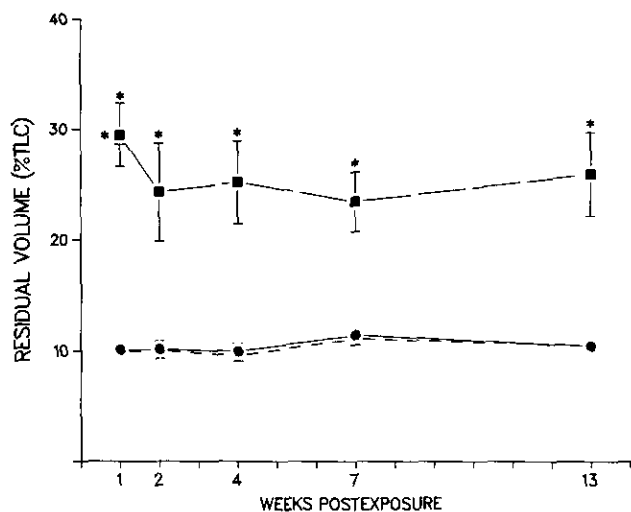


FIGURE 2. Residual volume as a percent of total lung capacity in rats exposed to (●) 0 ppm, (○) 3 ppm, (■) 10 ppm, or 30 ppm (results identical to rats exposed to 10 ppm at 1 week) MIC for 2 hr. The asterisk (*) denotes significant difference ($p < 0.05$) from control.

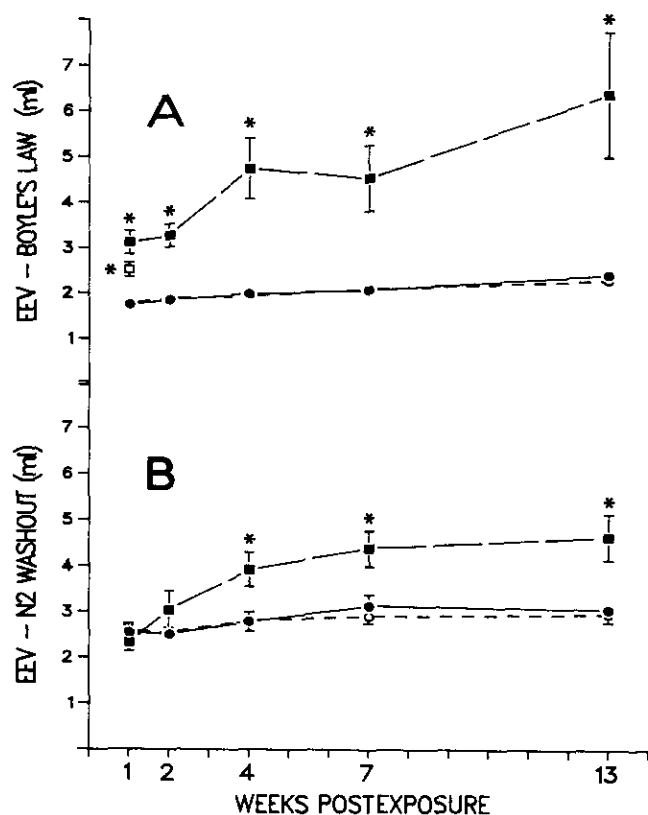


FIGURE 3. (A) End expiratory volume as determined by Boyle's Law and (B) expiratory volume as determined by nitrogen washout in rats exposed to (●) 0 ppm, (○) 3 ppm, (■) 10 ppm, or 30 ppm (results identical to rats exposed to 10 ppm at 1 week) MIC for 2 hr. The asterisk (*) denotes significant difference ($p < 0.05$) from control.

in both groups, DL_{co} was unchanged from control through 13 weeks. Although it appears that the diffusing capacity of the lung returned to control values, this recovery coincided with the abnormal increase of TLC. As a result, the diffusing capacity/unit lung volume (specific diffusing capacity) remained depressed at 17% below control in the rats exposed 10-ppm throughout the entire experiment (Fig. 4). This suggests that the rats exposed to 10 ppm MIC were able to maintain overall gas-diffusing homeostasis by increasing lung volumes and, probably, the correlated lung surface area. This phenomenon of elevating lung volumes to maintain gas exchange has been demonstrated in rats after inhalation exposure to volcanic ash (9), in rats 1 year after intratracheal administration of bleomycin (10) and in rats exposed subchronically to acrolein (11). Additionally, young rats exposed to a hypoxic environment, where available oxygen would be initially inadequate, exhibited elevated TLCs similar to the increases we presently report (12). These authors also morphometrically demonstrated an increase in the size and/or number of alveolar structures. Another study of hypoxia showed that comparable increases in TLC resulted in elevated DL_{co} values (13). Whether a similar mechanism is involved with increasing TLCs in the MIC-exposed animals, however, is unknown. Morphometric evaluations of these lungs will be performed to address this possibility more adequately.

Measurements of intrapulmonary gas distribution, as determined by the slope of the nitrogen washout curve, indicated marked dysfunction in the efficiency of ventilation distribution in the animals exposed to 10 and 30 ppm. However, this measure may have been influenced by the large increases in EEV observed in these rats. To adjust for this phenomenon, nitrogen washout curves were reanalyzed using the slope of expired nitrogen

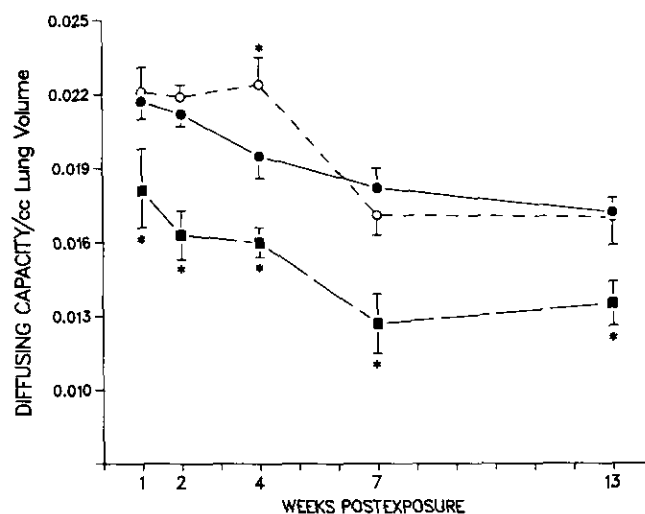


FIGURE 4. Lung diffusing capacity of carbon monoxide normalized to total lung capacity (in cm^3) in rats exposed to (●) 0 ppm, (○) 3 ppm, (■) 10 ppm, or 30 ppm (results identical to rats exposed to 10 ppm at 1 week) MIC for 2 hr. The asterisk (*) denotes significant difference ($p < 0.05$) from control.

concentration vs. the cumulative expired volume normalized to EEV (CEV slope). The results of this analysis revealed that rats exposed to 10 ppm MIC exhibited a consistent 40% depression in CEV slope through the entire 13 weeks after exposure (Fig. 5), indicating inefficient communication between lung compartments and subsequent inhomogeneous distribution of ventilated air. One explanation for this observation is the presence of trapped intrapulmonary air beyond damaged airways.

We indirectly tested for the presence of trapped gas in the lung by comparing the measure of EEV calculated by Boyle's Law to the EEV derived during the nitrogen washout analysis. The Boyle's Law method computes EEV based on pressure and volume changes, and accounts for all of the gas present in the thorax at the time of the measurement. The nitrogen washout method is based on the dilution of nitrogen and is most accurate when there is unobstructed communication between the airways and the nitrogen analyzer. Air trapped beyond an obstruction would not be included in this calculation, so the actual EEV would be underestimated. A comparison of Figures 3A and 3B illustrates this effect. At each time point, EEV in the animals receiving 10 ppm exposure was consistently higher when calculated by the Boyle's Law method than it was if the nitrogen washout method was used.

Given the highly reactive and water-soluble nature of MIC, a reasonable explanation for the trapped air in the lungs of these animals is an airway obstruction related to bronchiolar inflammation and scarring. The increased lung volume, a secondary effect, resulted possibly as an attempt to maintain adequate gas exchange. Consistent with this airway lesion hypothesis is the increase in average expiratory times measured during resting, tidal breathing. Although total breath and inspiratory times were unchanged with MIC exposure,

the symmetry of breathing was altered in the animals exposed to 10 ppm, so that expiration was consistently prolonged by an average to 10 to 20% throughout the course of the experiment. Continued evidence of airway impairment is seen 6 months after exposure to 10 ppm MIC where forced expiratory flows, a sensitive index of airway dysfunction, are significantly reduced (14).

Widespread parenchymal destruction, with resultant abnormal intrapulmonary spaces, could be an alternative explanation for the presence of trapped gas, depressed specific diffusing capacities, and increased lung volumes. However, histologic examination of lung tissue from the rats exposed to 10 ppm indicated very little alveolar impairment, while the most pronounced damage was manifest as initial, complete destruction of airway epithelium (15).

The distensibility of the lung, indicated by C_{rs} , was depressed by 24% in the rats exposed to 30 ppm and 9% in the rats exposed to 10 ppm when compared to control at 1 week after exposure, but was unchanged from control throughout the remaining 13 weeks. Volume-specific compliance (C_{rs}/mL), however, was consistently depressed by 25% at all time points in the animals exposed to 10 ppm (Fig. 6). This is a reflection of the tremendous increase in RV in these animals and may indicate that portions of the lung were not contributing to the compliance measurement.

Both wet and dry lung weights of the rats exposed to 10 ppm were significantly elevated above control during the 13 weeks after exposure (Table 1). The relatively small increase in percent lung water (approximately 2%) rules out the presence of widespread edema in these lungs. Regression analysis reveals that the lung weights of these animals increased at a higher rate than those of control rats throughout the 13-week study period. This may indicate progressive hypercellularity in the lungs of these animals due to chronic inflammatory re-

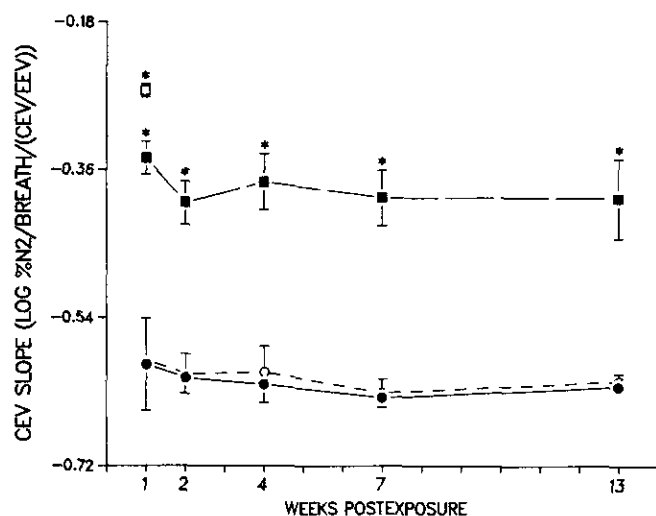


FIGURE 5. Slope of the multibreath nitrogen washout curve normalized for changes in EEV (see discussion) in rats exposed to (●) 0 ppm, (○) 3 ppm, (■) 10 ppm, or (□) 30 ppm MIC for 2 hr. The asterisk (*) denotes significant difference ($p < 0.05$) from control.

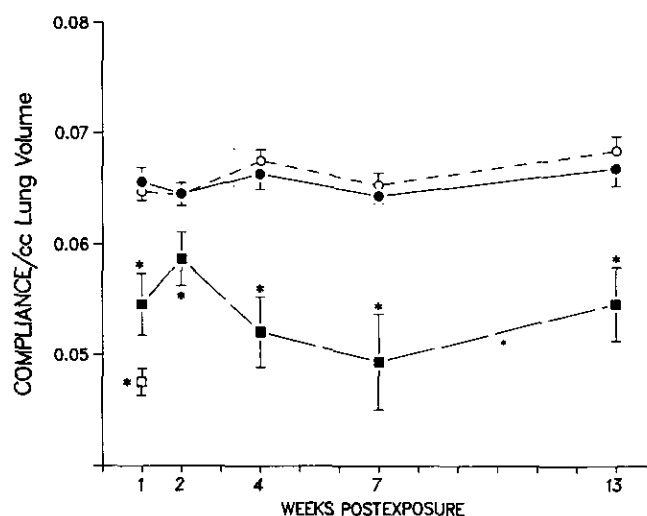


FIGURE 6. Respiratory system compliance normalized to total lung capacity (in cm^3) in rats exposed to (●) 0 ppm, (○) 3 ppm, (■) 10 ppm, or (□) 30 ppm MIC for 2 hr. The asterisk (*) denotes significant difference ($p < 0.05$) from control.

Table 1. Mean wet and dry lung weights and percent lung water (\pm SEM) in rats 1, 2, 4, 7, and 13 weeks after a single 2-hr exposure to 0, 3, 10, or 30 ppm MIC.

Time after exposure, weeks	Measurement	MIC exposure concentration			
		0 ppm	3 ppm	10 ppm	30 ppm
1	Lung wet, g	0.845 \pm 0.017	0.837 \pm 0.011	0.976 \pm 0.061*	0.830 \pm 0.044
	Lung dry, g	0.194 \pm 0.004	0.192 \pm 0.003	0.218 \pm 0.011	0.186 \pm 0.011
	Lung H ₂ O, %	77.10 \pm 0.27	77.09 \pm 0.14	77.63 \pm 0.34	77.63 \pm 0.34
2	Lung wet, g	0.861 \pm 0.028	0.878 \pm 0.019	1.164 \pm 0.044*	—
	Lung dry, g	0.208 \pm 0.008	0.213 \pm 0.006	0.253 \pm 0.008*	—
	Lung H ₂ O, %	76.10 \pm 0.38	75.64 \pm 0.89	78.19 \pm 0.51*	—
4	Lung wet, g	0.975 \pm 0.022	0.968 \pm 0.023	1.315 \pm 0.066*	—
	Lung dry, g	0.236 \pm 0.008	0.239 \pm 0.007	0.297 \pm 0.010*	—
	Lung H ₂ O, %	75.82 \pm 0.26	75.35 \pm 0.32	77.29 \pm 0.51*	—
7	Lung wet, g	1.043 \pm 0.022	1.008 \pm 0.024	1.360 \pm 0.058*	—
	Lung dry, g	0.262 \pm 0.005	0.256 \pm 0.007	0.321 \pm 0.001*	—
	Lung H ₂ O, %	73.82 \pm 0.24	74.59 \pm 0.27	76.23 \pm 0.36*	—
13	Lung wet, g	1.218 \pm 0.030	1.138 \pm 0.034	1.701 \pm 0.156*	—
	Lung dry, g	0.319 \pm 0.009	0.289 \pm 0.010	0.403 \pm 0.033*	—
	Lung H ₂ O, %	73.82 \pm 0.24	74.59 \pm 0.27	76.23 \pm 0.36*	—

*Significant difference ($p < 0.05$) from control.

sponse or, perhaps, partly to repair and/or the addition of new lung tissue. The significance of this latter type of response to severe lung injury in the rodent as it relates to that in humans is not presently clear. Taken collectively with the data that suggested that the rodent lung was able to compensate functionally to the severe MIC injury and maintain adequate gas exchange, this increased lung weight may suggest that new lung tissue, as well as hyperinflation, is involved in the rat's response to the MIC-induced damage. No direct data addressing this question are currently available.

We present data that demonstrate severely compromised pulmonary function in rats after a 2-hr exposure to 10 ppm MIC. The functional lesion resulting from this exposure does not appear to be associated primarily with the alveoli, as the animals are able to maintain normal alveolar function by increasing maximal lung volume via an unknown mechanism. Gas trapping is apparent in the rats exposed to 10 ppm, as indicated by the inhomogeneous distribution of intrapulmonary air and the marked differences in EEV measured by the Boyle's Law or nitrogen washout method. Additionally, increased expiratory times in the rats exposed to 10 ppm and depressed forced expiratory flows 6 months after exposure offer indirect evidence of airway obstruction (14).

In conclusion, a pronounced concentration-response curve appears to exist between the 3-ppm and 10-ppm MIC exposures. No abnormal functional deficits were observed at 3 ppm, while exposure to 10 ppm MIC resulted in an obstructive airway lesion without evidence of recovery after 13 weeks.

The research described in this article has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

REFERENCES

1. Kimmerle, G., and Eben, A. Toxicity of methyl isocyanate and how to determine its quantity in air. *Arch. Toxikol.* 20: 235-241 (1964).
2. Pozzani, V. C., and Kinkead, E. R. Animal and human response to methyl isocyanate. Paper presented at the annual meeting of the American Industrial Hygiene Association. Pittsburgh, PA, May 16-20, 1966.
3. Ferguson, J. S., Schaper, M., Stock, M. F., Weyel, D. A., and Alarie, Y. Sensory and pulmonary irritation with exposure to methyl isocyanate. *Toxicol. Appl. Pharmacol.* 82: 329-335 (1986).
4. Kamat, S. R., Mahashur, A. A., Tiwari, A. K. B., Potdar, P. V., Gaur, M., Kolhatkar, V. P., Vaidya, P., Parmar, D., Rupwate, R., Chatterjee, T. S., Jain, K., Kelkar, M. D., and Kinare, S. G. Early observations on pulmonary changes and clinical morbidity due to the isocyanate gas leak at Bhopal. *J. Postgrad. Med.* 31 (2): 63-72 (1985).
5. Adkins, B., Jr., O'Connor, R. W., and Dement, J. M. Inhalation exposure system used for acute and repeated-dose methyl isocyanate exposures of laboratory animals. *Environ. Health Perspect.* 72: 45-51 (1987).
6. Raub, J. A., Mercer, R. R., Miller, F. J., Graham, J. A., and O'Neil, J. J. Dose response of elastase-induced emphysema in hamsters. *Am. Rev. Respir. Dis.* 125: 432-435 (1982).
7. Raub, J. A., Miller, F. J., Graham, J. A., Gardner, D. E., and O'Neil, J. J. Pulmonary function in normal and elastase-treated hamsters exposed to a complex mixture of olefin-ozone-sulfur dioxide reaction products. *Environ. Res.* 31: 302-310 (1983).
8. Takezawa, J., Miller, F. J., and O'Neil, J. J. Single-breath diffusing capacity and lung volumes in small laboratory mammals. *J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.* 48(6): 1052-1059 (1980).
9. Raub, J. A., Hatch, G. E., Mercer, R. R., Grady, M., and Hu, P. C. Inhalation studies of Mt. St. Helens volcanic ash in animals. II. Lung function, biochemistry, and histology. *Environ. Res.* 37: 72-83 (1985).
10. Costa, D. L., Lehmann, J. R., Slatkin, D. N., Popenoe, E. A., and Drew, R. T. Chronic airway obstruction and bronchiectasis in the rat after intratracheal bleomycin. *Lung* 161: 287-300 (1983).
11. Costa, D. L., Kutzman, R. S., Lehmann, J. R., and Drew, R. T. Altered lung function and structure in the rat after subchronic acrolein. *Am. Rev. Respir. Dis.* 133: 286-291 (1986).
12. Cunningham, E. L., Brody, J. S., and Jain, B. P. Lung growth induced by hypoxia. *J. Appl. Physiol.* 37(3): 362-366 (1974).
13. Costa, D. L., Lehmann, J. R., Osheroff, M. R., and Bergofsky, E. H. Lung enlargement in rats after chronic hypoxia (abstract). *Am. Rev. Respir. Dis.* 129 (Part 4:2): A211 (1984).

14. Tepper, J. S., Costa, D. L., Weister, M. J., and Weber, M. F. Cardiopulmonary measurements in awake rats after MIC exposure. *Environ. Health Perspect.* 72: 95-103 (1987).
15. Bucher, J. R., Boorman, G. A., Gupta, B. N., Uriah, L. C., Hall, L. B., and Stefanski, S. A. Two-hour methylisocyanate exposure and 91-day recovery: a preliminary description of pathologic changes in F344 rats. *Environ. Health. Perspect.* 72: 71-75 (1987).